



(12) **EUROPEAN PATENT APPLICATION**

(88) Date of publication A3:
31.03.2004 Bulletin 2004/14

(51) Int Cl.⁷: **C12Q 1/68**

(43) Date of publication A2:
04.12.2002 Bulletin 2002/49

(21) Application number: **02016384.6**

(22) Date of filing: **06.01.1994**

(84) Designated Contracting States:
**AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL
PT SE**

(30) Priority: **07.01.1993 US 1323**

(62) Document number(s) of the earlier application(s) in
accordance with Art. 76 EPC:
94906047.9 / 0 679 196

(71) Applicant: **SEQUENOM, INC.**
San Diego, California 92121 (US)

(72) Inventor: **Köster, Hubert**
6900 Lugano-Cassarate (CH)

(74) Representative: **Baldock, Sharon Claire et al**
BOULT WADE TENNANT,
Verulam Gardens
70 Gray's Inn Road
London WC1X 8BT (GB)

(54) **Dna sequencing by mass spectrometry**

(57) The invention describes a new method to sequence DNA. The improvements over the existing DNA sequencing technologies are high speed, high throughput, no electrophoresis and gel reading artifacts due to the complete absence of an electrophoretic step, and no costly reagents involving various substitutions with stable isotopes. The invention utilizes the Sanger sequencing strategy and assembles the sequence information by analysis of the nested fragments obtained by base-specific chain termination via their different molec-

ular masses using mass spectrometry, as for example, MALDI or ES mass spectrometry. A further increase in throughput can be obtained by introducing mass-modifications in the oligonucleotide primer, chain-terminating nucleoside triphosphates and/or in the chain-elongating nucleoside triphosphates, as well as using integrated tag sequences which allow multiplexing by hybridization of tag specific probes with mass-differentiated molecular weights.



European Patent
Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

EP 02 01 6384

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.5)
A	WO 92/13629 A (UNIV WAYNE STATE) 20 August 1992 (1992-08-20) * the whole document *	1-105	C12Q1/68
A	JACOBSON K B ET AL: "APPLICATIONS OF MASS SPECTROMETRY TO DNA SEQUENCING" GENETIC ANALYSIS TECHNIQUES AND APPLICATIONS, ELSEVIER SCIENCE PUBLISHING, NEW YORK, US, vol. 8, no. 8, 1 December 1991 (1991-12-01), pages 223-229, XP000271820 ISSN: 1050-3862 * the whole document *	1-105	
A	US 5 003 059 A (BRENNAN THOMAS M) 26 March 1991 (1991-03-26) * the whole document *	1-105	
A	WO 89/09282 A (HOLMES MICHAEL JOHN ;CEMU BIOTEKNIK (SE)) 5 October 1989 (1989-10-05) * the whole document *	1-105	
			TECHNICAL FIELDS SEARCHED (Int.Cl.5)
			C12Q
INCOMPLETE SEARCH			
<p>The Search Division considers that the present application, or one or more of its claims, does/do not comply with the EPC to such an extent that a meaningful search into the state of the art cannot be carried out, or can only be carried out partially, for these claims.</p> <p>Claims searched completely :</p> <p>Claims searched incompletely :</p> <p>Claims not searched :</p> <p>Reason for the limitation of the search:</p> <p>see sheet C</p>			
Place of search		Date of completion of the search	Examiner
Munich		21 October 2003	Wimmer, G
<p>CATEGORY OF CITED DOCUMENTS</p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document</p>			

EPO FORM 1503 (03.82) (P04C07)



European Patent
Office

**INCOMPLETE SEARCH
SHEET C**

Application Number
EP 02 01 6384

Claim(s) not searched:
47-62, 74-84

Reason for the limitation of the search:

Subject-matter of claims 47-62, 74-84 relates to species of ions. Not only is the material nature of such ions not clearly defined (e.g. an "ionized duplex" of undefined substance) in certain claims, but also are these ions defined by results to be achieved ("intact", "volatilized") rather than by clear technical terms. Also, the definition of "intact" and such features as "mass modified" lacks clarity. The claims therefore lack clarity and enablement to such an extent as to render a meaningful search of these claims impossible.



European Patent
Office

Application Number

EP 02 01 6384

CLAIMS INCURRING FEES

The present European patent application comprised at the time of filing more than ten claims.

- ☐ Only part of the claims have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims and for those claims for which claims fees have been paid, namely claim(s):
- ☐ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims.

LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

see sheet B

- ☐ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.
- ☐ As all searchable claims could be searched without effort justifying an additional fee, the Search Division did not invite payment of any additional fee.
- ☐ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid, namely claims:
- ☒ None of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims, namely claims:

1-27



European Patent
Office

LACK OF UNITY OF INVENTION
SHEET B

Application Number
EP 02 01 6384

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

1. claims: 1-27

A method of concurrent sequencing two or more nucleic acids by mass spectrometry of base-specifically terminated nucleic acid fragments, wherein the fragments of the individual nucleic acids are distinguishable by molecular weight.

2. claims: 28-46, 85, 86, 88-99

solid supports linked to a mass-modified nucleic acid primer or to an elongated mass modified nucleic acid, insofar as not limited to the use in the method of invention 1

3. claim: 87

A microtiter plate comprising a solid support and a reversibly linked nucleic acid primer

**ANNEX TO THE EUROPEAN SEARCH REPORT
ON EUROPEAN PATENT APPLICATION NO.**

EP 02 01 6384

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

21-10-2003

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9213629	A	20-08-1992	US 5210412 A	11-05-1993
			WO 9213629 A1	20-08-1992
			US 5580733 A	03-12-1996
US 5003059	A	26-03-1991	AU 632756 B2	14-01-1993
			AU 3847789 A	12-01-1990
			EP 0379548 A1	01-08-1990
			JP 3500093 T	10-01-1991
			WO 8912694 A1	28-12-1989
			US 5174962 A	29-12-1992
WO 8909282	A	05-10-1989	AT 91156 T	15-07-1993
			AU 633252 B2	28-01-1993
			AU 3360289 A	16-10-1989
			DE 68907407 D1	05-08-1993
			DE 68907407 T2	18-11-1993
			DE 406296 T1	11-06-1992
			EP 0406296 A1	09-01-1991
			WO 8909282 A1	05-10-1989
			JP 4500902 T	20-02-1992
			JP 3068144 B2	24-07-2000
			NO 904133 A	21-11-1990
			US 5405746 A	11-04-1995